

US EPA ARCHIVE DOCUMENT

TITLES: REVISED CONFIRMATORY PROCEDURE FOR THE DETERMINATION OF COBRA™  
AND METABOLITE RESIDUES IN SOYBEAN SEED

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EPA DATA  
REQUIREMENT: 158 PART 125 171-4  
ANALYTICAL METHODS

# CONFIDENTIALITY STATEMENT

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA § 10 (d) (1) (A), (B), or (C).

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## **I. Introduction**

PPG-844, also known as Cobra<sup>TM</sup>, or 1-(Carboethoxy) ethyl 5-[2-chloro-4-(trifluoromethyl) phenoxy]-2-nitrobenzoate is an experimental herbicide that has shown promise for the control of broadleaved weeds when applied postemergent to soybeans at a rate of 0.1-0.2 lb/A. A hydrolysis procedure has been developed<sup>1</sup> which converts all potential soybean metabolites to two components representing the nitro DPE residue and amino DPE residue for analysis. Since both of these residues are potentially composed of two or more compounds, a specific method for each compound is a useful confirmatory procedure. This method was designed to measure PPG-844 and each of these individual metabolites: PPG-1576, PPG-947, PPG-847, PPG-2597.

During the method trial conducted by EPA scientists at The Barberton Technical Center on March 2 and 3, 1987, several modifications and clarifications to this method were suggested to enhance its performance. This report describes this method in detail, incorporating these modifications.

## **II. Abstract**

A method is described for use in determining residues of PPG-844 and four metabolites, PPG-847, PPG-947, PPG-1576 and PPG-2597 in soybeans. Briefly, these compounds are extracted with acetonitrile containing 1% triethylamine, methylated with diazomethane and purified by silica gel column chromatography. The residues are quantified by electron capture gas chromatography. Typically limits of detection achieved by this method are 0.01 ppm for each component.

The mean recovery for 0.01-0.24 ppm PPG-844 was 95.5% with a range of 63-158% and a standard deviation of 17.5%. Recoveries of PPG-1576, PPG-947, PPG-847 and PPG-2597 at approximately the same fortification levels had means of 84.8, 85.6, 87, and 66% respectively. Standard deviations were 19.0, 17.2, 18.3, and 8.1% respectively.

This procedure is rapid (two hours/sample analytical time and 4-1/2 hours/sample total time) and accurate so it should be suitable for use as a residue confirmatory procedure.

The method described incorporates several modifications and clarifications to BR-24085<sup>2</sup> suggested by EPA scientists during the method trial conducted at BTC on March 2,3, 1987.

III. Experimental (Notebooks 6358, pp. 22-130; 6611, pp. 2-56; 6465, pp. 96-212)

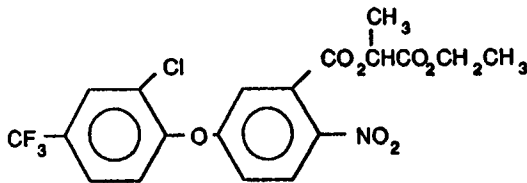
This procedure was designed specifically for soybean seed and was condensed from the more general procedure given in BR-23728<sup>3</sup>, thus, minor modifications may be required for some other matrices.

A. Apparatus

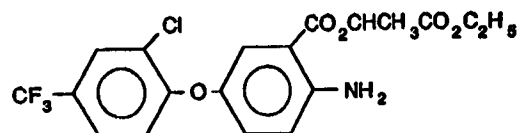
1. Rotary flash evaporator - Buchi (Brinkmann), or equivalent
2. Round-bottom flasks - 100 ml, 250 or 1000 ml, with single neck and 24/40 joint
3. Buchner Funnels - 12.5 and 5.5 cm
4. Filter Flasks - 500 ml and 50 or 125 ml
5. Blender - Waring, or equivalent, with 1 quart containers
6. Varian Model 3500 Gas Chromatograph equipped with a capillary inlet system and electron capture detector
7. Hewlett-Packard Model 3357 - Laboratory Automation System
8. Luxbeam Vista Professional Food Processor, or equivalent, with cutting/processing blade.
9. Chromatographic columns glass, 10 cm x 8 mm i.d., with 30 ml reservoir.
10. Centrifuge, Fisher Model 228 or equivalent.
11. Centrifuge tubes, 15 ml, Cat.#05-538-29A, Fisher Scientific Co.
12. Mini-Diazald Apparatus, Cat. #710889-8, Aldrich Chemical Co.

B. Reagents and Chemicals

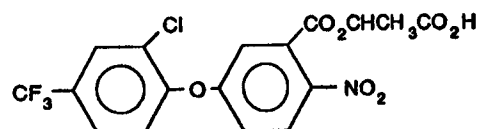
1. Solvents - acetonitrile, methylene chloride, both HPLC grade, J. T. Baker Chemical Co.; toluene, Baker "Resi-analyzed", J. T. Baker Chemical Co.; diethyl ether and ethanol, ACS reagent grade, Fisher Scientific Co.; hexane, pesticide grade, Fisher Scientific Co.; triethylamine, reagent grade, Fisher Scientific Co.
2. Diazald<sup>TM</sup>, Cat. #D2800-0, Aldrich Chemical Co.
3. Potassium Hydroxide, ACS reagent grade, Fisher Scientific Co.
4. Sodium Hydroxide, ACS reagent grade, Fisher Scientific Co.
5. Silica gel Activity I, Cat. #404727, ICN Nutritional Biochemicals Prepared for use by adding 20 g of water to each 100 g of silica gel. Container should be gently rotated to remove lumps and then allowed to equilibrate for twenty-four hours before use.
6. PPG-844, Sample No. 374-2743 PPG Industries, Inc., 100.9% assay



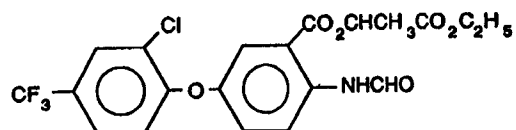
7. PPG-1576, Sample No. 374-2611, PPG industries, Inc., 95.59% assay



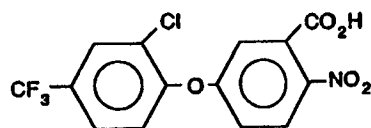
8. PPG-947, Sample No. 374-2645, PPG Industries, Inc., 96.6% assay



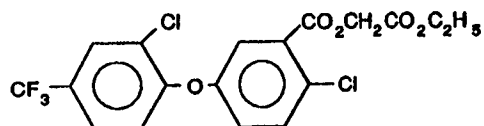
9. PPG-2597, Sample No. 374-2612, PPG Industries, Inc., 95.2% assay



10. PPG-847, Sample No. 314-5730, PPG Industries, Inc., 99.5% assay



11. PPG-1827, Sample No. 557-712, PPG Industries, Inc. (internal standard)



12. Methyl 2-[4-(2,4-dichlorophenoxy) phenoxy] propanoate (diclofop-methyl) (optional internal standard)

### C. Extraction of Samples

Representative 1-2 pound samples of soybean seed are thrashed and ground into meal. Representative 10 g samples of the meal are extracted as follows: samples are transferred to a blender jar, blended for one minute with 150 ml of acetonitrile containing 1% triethylamine (TEA) and vacuum filtered through Whatman #41 filter paper using a Buchner funnel. Filter cakes are returned to the blender jar, re-extracted with 150 ml of acetonitrile-TEA and filtered as above. The combined filtrates are rotary evaporated to dryness at 25 inch Hg vacuum and 50°C water bath temperature and the residues transferred to 10 ml volumetric flasks with ~ 5 ml of methylene chloride and diluted to volume with acetonitrile. Since soybean seed extracts contain excessive amounts of oil, they are further partitioned as follows: 1 ml of each extract (flask should be allowed to stand 15-30 minutes until any particulates present have settled or the extract may be briefly centrifuged) is evaporated to dryness and transferred to 15 ml centrifuge tubes using ~ 2 ml hexane and 5 ml of acetonitrile. The tubes are shaken and centrifuged for 2-3 minutes at 3300 rpm. The acetonitrile phase (bottom) is withdrawn using a 1 ml glass syringe and two inch needle, transferred to a 6 dram vial and evaporated to 0.5-1.0 ml with nitrogen (50°C water bath).

### D. Methylation of Extracts

Sample extracts were derivatized with diazomethane prepared using the Mini-Diazald<sup>TM</sup> apparatus<sup>4</sup>. One-half (0.5) ml of diazomethane solution was added to each extract in a 6 dram screw cap vial. Each mixture was capped and allowed to stand for 15 minutes before evaporating to dryness with a stream of nitrogen in a laboratory hood.

### E. Cleanup of Extracts by Silica Gel Chromatography

Chromatographic cleanup columns are packed to a height of 10 cm with 20% water deactivated silica gel with gentle tapping. Column beds are covered with ~1 g of sodium sulfate and pre-washed with 3-4 ml of eluting solvent, methylene chloride:hexane (70:30 v/v). The methylated sample extracts are quantitatively transferred to the silica gel columns using 5-1 ml aliquots of eluting solvent and eluted with an additional 25 ml (30 ml total collected) into 100 ml round bottom flasks. The eluants are then rotary-evaporated to dryness (50°C water bath) and transferred to 5 ml volumetric flasks with acetonitrile.

### F. Gas Chromatography

One ml of each "cleaned-up" extract is transferred to a 6 dram vial and 100 µl of a toluene solution containing 0.2 µg/ml of an internal standard is added (PPG-1827 or diclofop-methyl). The mixture is evaporated to dryness with a stream of nitrogen and a warm water bath and re-dissolved in 1 ml of toluene. One µl of this solution is injected into the chromatograph operating under the following conditions:

Column: 30M x 0.32 mm i.d. DB-5 (0.25 µm film thickness), J & W Scientific, Inc., Cat. #123-5032

### Temperatures:

Column: 80°C for 2 min. programmed to 230°C at 10°C/min.  
Hold at upper limit 50 minutes

Injection Port: Splitless mode, 200°C isothermal. Split vent opened  
0.5 min. after injection

Detector: 300°C

### Flow Rates:

Carrier Gas: He, 1.29 ml/min.  
Make-up: N<sub>2</sub>, 25 ml/min.

### Retention Times, min.:

PPG-847:	20.43
PPG-1827:	25.61 (Internal standard)
PPG-947:	28.15
PPG-1516:	28.84
PPG-844:	30.33 (lactofen)
PPG-2597:	36.62

### G. Standard Preparation

A standard solution is prepared in acetonitrile containing 0.1-0.2 ug/ml of each of the components to be determined. This solution is then used to "spike" untreated control samples prior to extraction for determination of method recovery. This solution is also used to prepare a GC calibration standard as follows: 1.0 ml of the above solution is transferred to a 6 dram screw cap vial and 0.5 ml of diazomethane solution is added. The vial is securely capped and allowed to stand for 15 minutes before evaporating to dryness with a stream of nitrogen in a laboratory hood. The residue is redissolved in toluene and transferred to a 50 ml volumetric flask containing a 5 ml aliquot of the internal standard solution used to prepare samples. This standard is then diluted to volume with toluene.

### H. Computation of Results

The amount of PPG-844 and each metabolite present in each sample extract is computed by the data system using the parameters shown in Appendix I. The calibration standard amounts are entered into the computer in ppb, calculated using the actual solution concentration, the sample weight analyzed and the final extract volume. For example, if the standard solution contains 3.12 ug/l of PPG-847, the sample weight analyzed is 1 g and final extract volume is 0.005 l, the concentration entered into the data system is 15.6 ppb  $[(3.12 \text{ ug/l} \times 0.005 \text{ l}) \div 1 \text{ g} = 0.0156 \text{ ug/g or } 15.6 \text{ ppb}]$ . The amount of each metabolite found may be expressed as PPG-844 equivalents by multiplying the amount found by the molecular weight ratio (e.g. 1.277 for PPG-847).



If no data system is available, standard and sample peak heights for each component can be measured manually and the amount of each component present in samples calculated using the following formula:

$$\text{ppm PPG-844} = \frac{P_i \times RF_i \times C_i}{S_{is}}$$

where  $P_i$  = peak height of component i  
 $S_{is}$  = peak height of internal standard  
 $RF_i$  = response factor for component i  
 $C_i$  = factor to convert to PPG-844 equivalent if desired.

$RF_i$  is determined from a standard chromatogram using the following formula:

$$RF_i = \frac{S_{is} \times C_i \times D}{P_i \times W}$$

where  $C_i$  = concentration of component i in the standard (ug/l)  
 $D$  = final extract volume (0.005 l)  
 $W$  = weight of the sample aliquot (1 g)

### I. Interferences

Because of the possibility of other pesticides being used with PPG-844, a survey was done to determine if any of these compounds would interfere with the method described. The following pesticides, which could potentially be used with PPG-844 were judged to have little or no response to electron capture and, therefore, would pose no interference problems: Basagran, Poast, Roundup, Sencor, Paraquat and Vernam. Treflan, and Lasso, two pesticides also used with PPG-844, were chromatographed to determine if they either had sufficient electron capture response or a retention time that would cause an interference problem. Both compounds showed detector response but the retention times were all less than 9 minutes and would, therefore, not interfere with the determination of PPG-844 or its metabolites. Several compounds with structural similarity to PPG-844 were chromatographed to determine if their retention times coincided with the retention time of any of the compounds determined by this method: Linuron, 2,4-D, Dinoseb, Dichlorprop, nitrofen, Hoelon, Modown and Fusilade. All of these compounds showed significant electron capture response except Fusilade (no peak was observed), but only nitrofen had a retention time that would interfere with this method. It was found to co-elute with PPG-847. Acifluorfen, which is the sodium salt of PPG-847 is a potential interference.

#### IV. Discussion

##### A. Safety<sup>2</sup>

Although quite safe when handled as a dilute solution in an inert solvent, diazomethane presents several safety hazards of which all users of the reagent should be aware. It is both extremely toxic and highly irritating, causing pulmonary edema when inhaled in high concentrations. Long-term, low-level exposure may lead to sensitization, resulting in asthma-like symptoms. Also, diazomethane and several of its chemical precursors have been cited as carcinogens.

Diazomethane has been known to explode quite unaccountably, both as a gas and as a liquid, although rough surfaces are proven initiators of detonations. Thus, ground-glass joints and any glassware which have not been carefully fire polished must never be allowed to come in contact with diazomethane or its solutions. In addition, contact with alkali metals or drying agents such as calcium sulfate can result in an explosion. If moisture must be removed from a solution containing diazomethane, the recommended drying agent is potassium hydroxide pellets. Finally, solutions should not be exposed to strong light, which has been reported to initiate detonations.

Fortunately, if the reagent is generated using the proper equipment and is handled only as a dilute solution at low temperature (ca. 0°C), the risks cited above are minimized. Of course, all reactions involving diazomethane should be carried out in an efficient fume hood and behind a sturdy safety shield. Finally, it is recommended that solutions of diazomethane be used immediately and not stored, even at low temperature.

##### B. Internal Standards

Using this method, results can be quantified using either internal or external standardization. All of the data reported here were obtained using internal standardization in order to minimize the effects of day to day instrument sensitivity variations and variations from one injection to another encountered by using a split injection technique. In choosing an internal standard, the following requirements should be taken into consideration:

- a. Must be completely resolved from any peaks of interest in the sample.
- b. Should elute near the peaks of interest.
- c. Must be similar in concentration and detector response to the peaks of interest.
- d. Should not react with any components of the sample.
- e. Must never be in the original sample.
- f. Should be of high purity and readily available.

The internal standard used in the method described, PPG-1827, was chosen because it met all of the above criteria except that it is not commercially available. Several commercially available pesticides such as linuron (3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea), dinoseb (2-sec-butyl-4,6-dinitrophenol), diclofop-methyl (methyl 2-[4-(2,4-dichlorophenoxy) phenoxy] propanoate), and bifenox (methyl 5-(2,4-dichlorophenoxy)-2-nitrobenzoate) were also found to meet the necessary requirements.

#### C. Detection Limit

The limit of detection of this method has been established to be 0.01 ppm for each component determined after careful consideration of two method parameters, sensitivity and matrix background.

Sensitivity is a measure of the ability of a detector, in this case, the electron capture detector, to signal a recorder or data system of the presence of an emerging sample component. The sensitivity, thus, determines the minimum amount of each component detectable, which is often defined as a peak which is twice as high as the noise level. While the sensitivity of this method varies from day to day and from compound to compound, the minimum amount of each component detectable has generally been 0.004 ppm. Two data system parameters, the slope sensitivity (mv/min) and minimum area are set to insure that "peaks" below this minimum are not reported. The slope sensitivity has been set at 0.3 mv/min. to reject low broad peaks and baseline changes, and the minimum area at 200 to prevent baseline noise from being integrated and reported.

The matrix background of the method has been established by surveying the analyses of control and soybean grain for PPG-844 to determine the typical response obtained. Although all responses were reported as <0.01 ppm, actual responses obtained ranged from 0.004 to 0.007. To ensure that these occasional matrix related responses would not be interpreted as residues, the limit of detection was set at twice this matrix background level or 0.01 ppm PPG-844. Similarly, 0.01 ppm detection limits were obtained for PPG-1576, PPG-947, PPG-847 and PPG-2597.

#### D. Recoveries

PPG-844 was added to soybean seed at levels between 0.011 and 0.24 ppm to provide recovery data by this procedure. Thirty-one samples had a mean recovery of 95.5%, a standard deviation of 17.5%, and a range of 63-158%. Recoveries of PPG-1576 were run from 0.012 ppm to 0.368 ppm. The mean of 22 analyses was 84.8% with a standard deviation of 19.0% and a range of 40-120%. The recovery of 0.015 to 0.228 ppm PPG-947 had a mean of 85.6%, a 17.2% standard deviation and a range of 61-130%. The recovery of PPG-847 from 22 soybean seeds had a mean of 97.0%, a standard deviation of 18.3%, and a range of 73-141%. The recovery of nine PPG-2597 analyses in soybean seed had a mean of 66%, a standard deviation of 8.1%, and a range of 48-75% at the 0.016 ppm fortification level. Tables 1 through 5 show the individual recovery data for these samples.

#### E. Modifications

The method described incorporates several modifications and clarifications suggested by EPA scientists during the method trial conducted at Barberton Technical Center including the use of a splitless injection technique for the analysis of samples. This change results in a significant improvement in the sensitivity of the method over the split injection technique originally used making quantitation of residues at the detection limit more accurate and reliable. This is, of course, due to the fact that the entire injection volume (1  $\mu$ l) reaches the column and detector with only the solvent tail being vented. Because of this increased sample loading the amount of internal standard added to samples and standards has been reduced by 1/10. Further reductions can be made if desired so internal standard amount more closely coincides with residue amounts being quantitated.

The acetonitrile - hexane partition used to remove excess oil from the sample extracts originally involved partitioning the entire extract with two aliquots of acetonitrile. This was simplified by partitioning only 1 ml of extract once with acetonitrile, resulting in a more efficient extraction. Centrifugation was also added to further improve the phase separation.

During the method trial conducted by EPA scientists at Barberton Technical Center, it was observed that the silica gel columns used were narrower in diameter than described in the original method. The method indicated 1 cm i.d. when columns were actually 1 cm o.d. Since the internal diameter was actually only 0.8 cm, much less silica gel is used for each column than would be if the method were followed as originally written. The description of the column was changed accordingly so that the volume of eluting solvent indicated would quantitatively elute the metabolites from the column.


PPG-2597 has been shown to be susceptible to decomposition during the GC analysis, probably due to active sites present in either the injection port or column. Replacing or appropriately deactivating the column and injection port liner may prevent the problem.

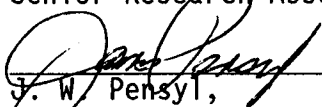
#### F. Chromatograms

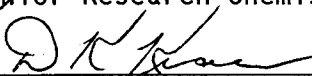
Representative chromatograms obtained by the described method are shown in Figures 1-4.

V. References

1. J. L. Wiedmann, J. W. Pensyl, BR-24080, 1/14/87.
2. J. W. Pensyl, J. L. Wiedmann, BR-24085 01/21/1987
3. J. L. Wiedmann, J. W. Pensyl, BR-23728, 07/01/85
4. T. Howard Black, Aldrichimica Acta, 16, 1, 1983.

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Table 1

## Recovery of PPG-844 From Soybean Seed Samples

Lab Sample No.	Plot Location	ppm PPG-844		Percent Recov'd.	Date Analyzed
		Added	Found		
C5017	C. Dowler-Tifton, GA	0	ND	---	04/03/84
C5017+S	C. Dowler-Tifton, GA	0.12	0.115	96	04/03/84
A8450	G. Jordan-Springfield, OH	0	ND	---	02/26/85
A8450+S	G. Jordan-Springfield, OH	0.12	0.122	102	02/26/85
A5224	PPG-Berry, KY	0	ND	---	03/15/85
A5224+S	PPG-Berry, KY	0.12	0.091	76	03/15/85
C4833	PPG-Barberton, OH	0	ND	---	03/13/85
C4833+S	PPG-Barberton, OH	0.12	0.189	153	03/13/85
C4447	G. Kapusta-Belleville, IL	0	ND	---	03/16/84
C4447+S	G. Kapusta-Belleville, IL	0.12	0.111	93	03/16/84
C4466	D. Guethle-Portageville, MO	0	ND	---	03/26/84
C4466+S	D. Guethle-Portageville, MO	0.12	0.126	105	03/26/84
C5396	Walker-Fairhope, AL	0	ND	---	03/05/84
C5396+S	Walker-Fairhope, AL	0.24	0.229	95	03/05/84
C7040	Fed. Univ.-Pelotas, RS, Brazil	0	ND	---	05/29/84
C7040+S	Fed. Univ.-Pelotas, RS, Brazil	0.12	0.098	82	05/29/84
C6259	Fac. Agron.-Jaboticabal, SP, Brazil	0	ND	---	05/31/84
C6259+S	Fac. Agron.-Jaboticabal, SP, Brazil	0.12	0.089	74	05/31/84
D0043	Fac. Agron.-Jaboticabal, SP, Brazil	0	ND	---	08/13/84
D0043+S	Fac. Agron.-Jaboticabal, SP, Brazil	0.12	0.117	98	08/13/84
C6264	Embrapa-Passo Fundo, RS, Brazil	0	ND	---	05/31/84
C6264+S	Embrapa-Passo Fundo, RS, Brazil	0.12	0.076	63	05/31/84
D0305	F. Exp. Station-Cruz Alta, Brazil	0	ND	---	09/05/84
D0305+S	F. Exp. Station-Cruz Alta, Brazil	0.12	0.108	90	09/05/84
C4701	PPG-Union, KY	0	ND	---	04/25/84
C4701+S	PPG-Union, KY	0.12	0.134	112	04/25/84

Table 1 (continued)

C-4638	W. Meggitt - E. Lansing, MI	0	ND	---	04/15/86
C-4638	W. Meggitt - E. Lansing, MI	0	ND	---	04/15/86
C-4638	W. Meggitt - E. Lansing, MI	0	ND	---	04/15/86
C-4638+S	W. Meggitt - E. Lansing, MI	0.0146	0.0119	81	04/15/86
C-4638+S	W. Meggitt - E. Lansing, MI	0.0146	0.0108	74	04/15/86
C-4638+S	W. Meggitt - E. Lansing, MI	0.0146	0.0138	94	04/15/86
C-5030	Banks - Athens, GA	0	ND	---	04/15/86
C-5030	Banks - Athens, GA	0	(0.0038) <sup>1</sup>	---	04/15/86
C-5030	Banks - Athens, GA	0	ND	---	04/15/86
C-5030+S	Banks - Athens, GA	0.0146	0.0141	96	04/15/86
C-5030+S	Banks - Athens, GA	0.0146	0.0138	85	04/15/86
C-5030+S	Banks - Athens, GA	0.0146	0.0125	85	04/15/86
C-4570	G. Harvey - Arlington, WI	0	ND	---	04/15/86
C-4570	G. Harvey - Arlington, WI	0	ND	---	04/15/86
C-4570	G. Harvey - Arlington, WI	0	(0.0036) <sup>1</sup>	---	04/15/86
C-4570+S	G. Harvey - Arlington, WI	0.0146	0.0119	81	04/15/86
C-4570+S	G. Harvey - Arlington, WI	0.0146	0.0110	75	04/15/86
C-4570+S	G. Harvey - Arlington, WI	0.0146	0.0126	86	04/15/86
C-4638	W. Meggitt - E. Lansing, MI	0	ND	---	08/19/85
C-4638	W. Meggitt - E. Lansing, MI	0	ND	---	08/19/85
C-4638	W. Meggitt - E. Lansing, MI	0	ND	---	08/19/85
C-4638+S	W. Meggitt - E. Lansing, MI	0.0109	0.0111	102	08/19/85
C-4638+S	W. Meggitt - E. Lansing, MI	0.0109	0.0106	99	08/19/85
C-4638+S	W. Meggitt - E. Lansing, MI	0.0109	0.0115	106	08/19/85
C-7903	D. Staniforth - Ames, IA	0	ND	---	08/19/85
C-7903	D. Staniforth - Ames, IA	0	ND	---	08/19/85
C-7903	D. Staniforth - Ames, IA	0	ND	---	08/19/85
C-7903+S	D. Staniforth - Ames, IA	0.0109	0.0120	111	08/19/85
C-7903+S	D. Staniforth - Ames, IA	0.0109	0.0111	102	08/19/85
C-7903+S	D. Staniforth - Ames, IA	0.0109	0.0114	105	08/19/85
A-9095	E. Beason - Elk City, KS	0	ND	---	08/19/85
A-9095	E. Beason - Elk City, KS	0	ND	---	08/19/85
A-9095	E. Beason - Elk City, KS	0	ND	---	08/19/85
A-9095+S	E. Beason - Elk City, KS	0.0109	0.0107	98	08/19/85
A-9095+S	E. Beason - Elk City, KS	0.0109	0.0121	111	08/19/85
A-9095+S	E. Beason - Elk City, KS	0.0109	0.0126	116	08/19/85

Soybean Seed Samples Recoveries: 31 Mean: 95.5 Std. Dev.: 17.5  
Range: 63-158

1. In order to provide data to estimate the matrix background the actual responses measured for control samples in the recovery trials are reported in parentheses. Results that did not exceed the minimum area are reported as N.D. Actual measured values (uncorrected) are reported for all spiked samples in the found column.

Table 2

## Recovery of PPG-1576 From Soybean Seed Samples

Lab Sample No.	Plot Location	ppm PPG-1576		Percent Recov'd.	Date Analyzed
		Added	Found		
C7040	Fed. Univ.-Pelotas, RS, Brazil	0	ND	---	05/29/84
C7040+S	Fed. Univ.-Pelotas, RS, Brazil	0.184	0.15	82	05/29/84
C6259	Fac. Agron.-Jaboticabal, SP, Brazil	0	ND	---	05/31/84
C6259+S	Fac. Agron.-Jaboticabal, SP, Brazil	0.184	0.14	76	05/31/84
D0043	Fac. Agron.-Jaboticabal, SP, Brazil	0	ND	---	08/13/84
D0043+S	Fac. Agron.-Jaboticabal, SP, Brazil	0.184	0.155	84	08/13/84
C6264	Embrapa-Passo Funco, RS, Brazil	0	ND	---	05/31/84
C6264+S	Embrapa-Passo Fundo, RS, Brazil	0.184	0.126	68	05/31/84
D0305	F. Exp. Station-Cruz Alta, Brazil	0	ND	---	09/05/84
D0305+S	F. Exp. Station-Cruz Alta, Brazil	0.184	0.169	92	09/05/84
C4701	PPG-Union, KY	0	ND	---	04/25/84
C4701+S	PPG-Union, KY	0.184	0.2	109	04/25/84
C5017	C. Dowler-Tifton, GA	0	ND	---	04/03/84
C5017+S	C. Dowler-Tifton, GA	0.184	0.198	108	04/03/84
A8450	G. Jordan-Springfield, OH	0	ND	---	02/26/85
A8450+S	G. Jordan-Springfield, OH	0.184	0.184	100	02/26/85
A5224	PPG-Berry, KY	0	ND	---	03/15/85
A5224+S	PPG-Berry, KY	0.184	0.144	78	03/15/85
C4833	PPG-Barberton, OH	0	ND	---	03/13/85
C4833+S	PPG-Barberton, OH	0.184	0.22	120	03/13/85
C4447	G. Kapusta-Belleleville, IL	0	ND	---	03/16/84
C4447+S	G. Kapusta-Belleleville, IL	0.184	0.12	65	03/16/84
C4466	D. Guethle-Portageville, MO	0	ND	---	03/26/84
C4466+S	D. Guethle-Portageville, MO	0.184	0.187	102	03/26/84
C5396	Walker-Fairhope, AL	0	ND	---	03/05/84
C5396+S	Walker-Fairhope, AL	0.3768	0.428	116	03/05/84
C-4638	E. Lansing, MI	0	ND	---	
C-4638	E. Lansing, MI	0	ND	---	
C-4638	E. Lansing, MI	0	ND	---	
C-4638+S	E. Lansing, MI	0.0121	0.0098	81	
C-4638+S	E. Lansing, MI	0.0121	0.0106	88	
C-4638+S	E. Lansing, MI	0.0121	0.0102	85	



Table 2 (continued)

C-5030	Athens, GA	0	ND	---
C-5030	Athens, GA	0	ND	---
C-5030	Athens, GA	0	ND	---
C-5030+S	Athens, GA	0.0121	0.0080	67
C-5030+S	Athens, GA	0.0121	0.0103	85
C-5030+S	Athens, GA	0.0121	0.0098	81
C-4570	Arlington, WI	0	ND	---
C-4570	Arlington, WI	0	ND	---
C-4570	Arlington, WI	0	ND	---
C-4570+S	Arlington, WI	0.0121	0.0087	72
C-4570+S	Arlington, WI	0.0121	0.0048	40
C-4570+S	Arlington, WI	0.0121	0.0079	66

Soybean Seed Samples      Recoveries: 22      Mean: 84.8      Std. Dev.: 19  
    Range: 40-120

Table 3

## Recovery of PPG-947 From Soybean Seed Samples

Lab Sample No.	Plot Location	ppm PPG-947		Percent Recov'd.	Date Analyzed
		Added	Found		
C5017	C. Dowler-Tifton, GA	0	ND	---	04/03/84
C5017+S	C. Dowler-Tifton, GA	0.114	0.113	99	04/03/84
A8450	G. Jordan-Springfield, OH	0	ND	---	02/26/85
A8450+S	G. Jordan-Springfield, OH	0.122	0.177	96	02/26/85
A5224	PPG-Berry, KY	0	ND	---	03/15/85
A5224+S	PPG-Berry, KY	0.122	0.08	66	03/15/85
C4833	PPG-Barberton, OH	0	ND	---	03/13/85
C4833+S	PPG-Barberton, OH	0.114	0.148	130	03/13/85
C4447	G. Kapusta-Belleville, IL	0	ND	---	03/16/84
C4447+S	G. Kapusta-Belleville, IL	0.114	0.107	94	03/16/84
C4466	D. Guethle-Portageville, MO	0	ND	---	03/26/84
C4466+S	D. Guethle-Portageville, MO	0.114	0.101	89	03/26/84
C5396	Walker-Fairhope, AL	0	ND	---	03/05/84
C5396+S	Walker-Fairhope, AL	0.228	0.231	101	03/05/84
C7040	Fed. Univ.-Pelotas, RS, Brazil	0	ND	---	05/29/84
C7040+S	Fed. Univ.-Pelotas, RS, Brazil	0.114	0.058	51	05/29/84
C6259	Fac. Agron.-Jaboticabal, SP, Brazil	0	ND	---	05/31/84
C6259+S	Fac. Agron.-Jaboticabal, SP, Brazil	0.114	0.07	61	05/31/84
D0043	Fac. Agron.-Jaboticabal, SP, Brazil	0	ND	---	08/13/84
D0043+S	Fac. Agron.-Jaboticabal, SP, Brazil	0.122	0.127	104	08/13/84
C6264	Embrapa-Passo Fundo, RS, Brazil	0	ND	---	05/31/84
C6264+S	Embrapa-Passo Fundo, RS, Brazil	0.114	0.076	67	05/31/84
D0305	F. Exp. Station-Cruz Alta, Brazil	0	ND	---	09/05/84
D0305+S	F. Exp. Station-Cruz Alta, Brazil	0.122	0.12	98	09/05/84
C4701	PPG-Union, KY	0	ND	---	04/25/84
C4701+S	PPG-Union, KY	0.114	0.113	99	04/25/84
C-4638	E. Lansing, MI	0	ND	---	
C-4638	E. Lansing, MI	0	ND	---	
C-4638	E. Lansing, MI	0	ND	---	
C-4638+S	E. Lansing, MI	0.0158	0.0118	75	
C-4638+S	E. Lansing, MI	0.0158	0.0128	82	
C-4638+S	E. Lansing, MI	0.0158	0.0137	87	

Table 3 (continued)

C-5030	Athens, GA	0	ND	---
C-5030	Athens, GA	0	ND	---
C-5030	Athens, GA	0	ND	---
C-5030+S	Athens, GA	0.0158	0.0135	86
C-5030+S	Athens, GA	0.0158	0.0133	84
C-5030+S	Athens, GA	0.0158	0.0131	87
C-4570	Arlington, WI	0	ND	---
C-4570	Arlington, WI	0	ND	---
C-4570	Arlington, WI	0	ND	---
C-4570+S	Arlington, WI	0.0158	0.0123	78
C-4570+S	Arlington, WI	0.0158	0.0120	76
C-4570+S	Arlington, WI	0.0158	0.0117	74

Soybean Seed Samples      Recoveries: 22      Mean: 85.6      Std. Dev.: 17.2  
    Range: 61-130

Table 4

## Recovery of PPG-847 From Soybean Seed Samples

Lab Sample No.	Plot Location	ppm PPG-847		Percent Recov'd.	Date Analyzed
		Added	Found		
C5017	C. Dowler-Tifton, GA	0	ND	---	04/03/84
C5017+S	C. Dowler-Tifton, GA	0.16	0.179	112	04/03/84
A8450	G. Jordan-Springfield, OH	0	(0.002) <sup>1</sup>	--	02/26/85
A8450+S	G. Jordan-Springfield, OH	0.16	0.147	91	02/26/85
A5224	PPG-Berry, KY	0	ND	---	03/15/85
A5224+S	PPG-Berry, KY	0.16	0.121	76	03/15/85
C4833	PPG-Barberton, OH	0	ND	---	03/13/85
C4833+S	PPG-Barberton, OH	0.16	0.184	115	03/13/85
C4447	G. Kapusta-Belleville, IL	0	ND	---	03/16/84
C4447+S	G. Kapusta-Belleville, IL	0.16	0.172	108	03/16/84
C4466	D. Guethle-Portageville, MO	0	ND	---	03/26/84
C4466+S	D. Guethle-Portageville, MO	0.16	0.176	110	03/26/84
C5396	Walker-Fairhope, AL	0	(0.005) <sup>1</sup>	--	03/05/84
C5396+S	Walker-Fairhope, AL	0.32	0.315	97	03/05/84
C7040	Fed. Univ.-Pelotas, RS, Brazil	0	ND	---	05/29/84
C7040+S	Fed. Univ.-Pelotas, RS, Brazil	0.16	0.148	93	05/29/84
C6259	Fac. Agron.-Jaboticabal, SP, Brazil	0	ND	---	05/31/84
C6259+S	Fac. Agron.-Jaboticabal, SP, Brazil	0.16	0.226	141	05/31/84
D0043	Fac. Agron.-Jaboticabal, SP, Brazil	0	ND	---	08/13/84
D0043+S	Fac. Agron.-Jaboticabal, SP, Brazil	0.16	0.172	109	08/13/84
C6264	Embrapa-Passo Fundo, RS, Brazil	0	ND	---	05/31/84
C6264+S	Embrapa-Passo Fundo, RS, Brazil	0.16	0.197	123	05/31/84
D0305	F. Exp. Station-Cruz Alta, Brazil	0	ND	---	09/05/84
D0305+S	F. Exp. Station-Cruz Alta, Brazil	0.16	0.151	94	09/05/84
C4701	PPG-Union, KY	0	ND	---	04/25/84
C4701+S	PPG-Union, KY	0.16	0.196	123	04/25/84
C-4638	E. Lansing, MI	0	ND	---	
C-4638	E. Lansing, MI	0	ND	---	
C-4638	E. Lansing, MI	0	ND	---	
C-4638+S	E. Lansing, MI	0.0125	0.0105	85	
C-4638+S	E. Lansing, MI	0.0125	0.0099	79	
C-4638+S	E. Lansing, MI	0.0125	0.0103	83	

Table 4 (continued)

C-5030	Athens, GA	0	ND	---
C-5030	Athens, GA	0	ND	---
C-5030	Athens, GA	0	ND	---
C-5030+S	Athens, GA	0.0125	0.0110	88
C-5030+S	Athens, GA	0.0125	0.0115	93
C-5030+S	Athens, GA	0.0125	0.0110	88
C-4570	Arlington, WI	0	ND	---
C-4570	Arlington, WI	0	ND	---
C-4570	Arlington, WI	0	ND	---
C-4570+S	Arlington, WI	0.0125	0.0096	77
C-4570+S	Arlington, WI	0.0125	0.0095	76
C-4570+S	Arlington, WI	0.0125	0.0092	73

Soybean Seed Samples      Recoveries: 22      Mean: 97.0      Std. Dev.: 18.3  
    Range: 73-141

1. In order to provide data to estimate the matrix background the actual responses measured for control samples in the recovery trials are reported in parentheses. Results that did not exceed the minimum area are reported as N.D. Actual measured values (uncorrected) are reported for all spiked samples in the found column.

Table 5

## Recovery of PPG-2597 From Soybean Seed Samples

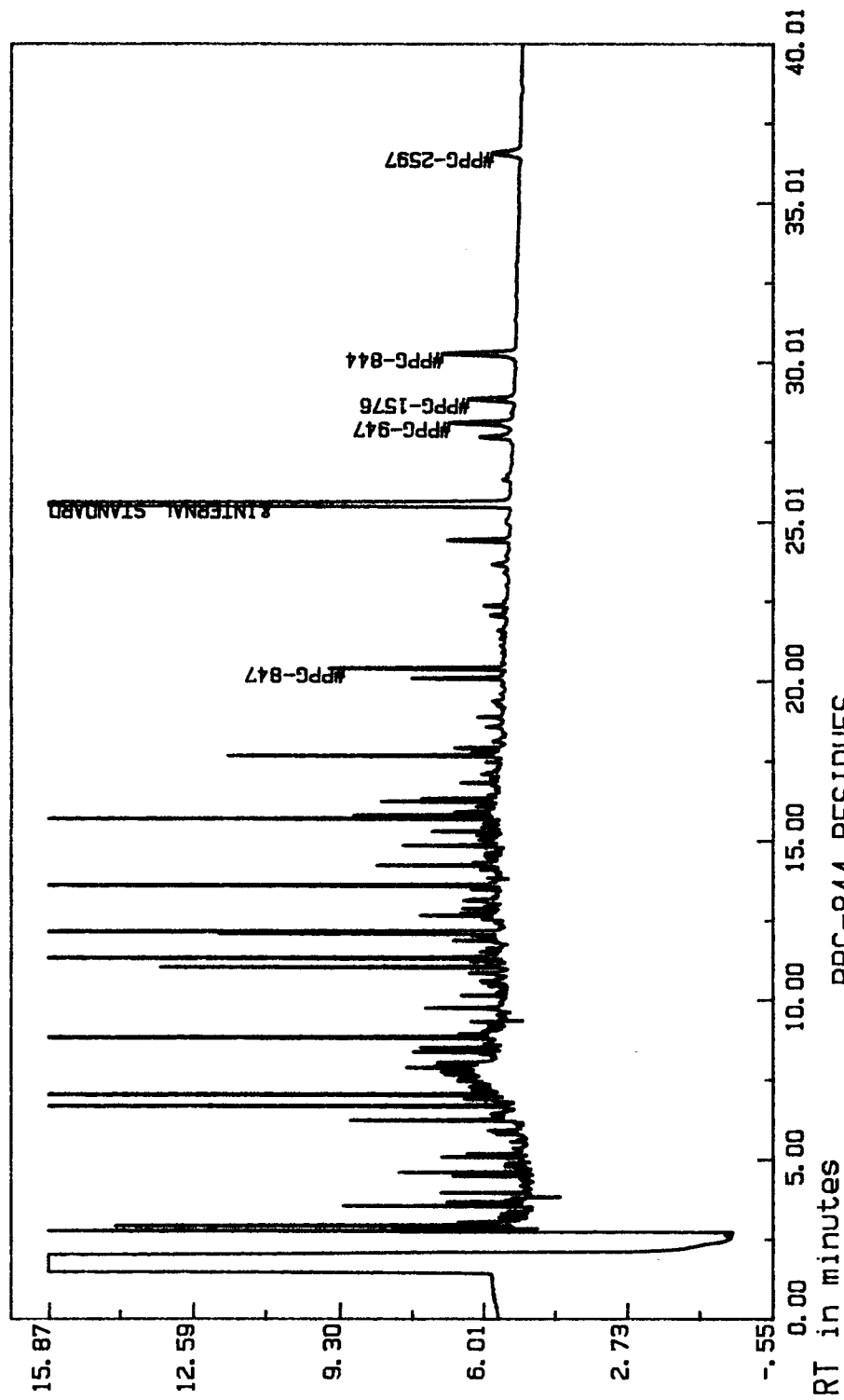
<u>Sample No.</u>	<u>Added</u>	<u>Found</u>	<u>% Recovery</u>
C-5030 <sup>1</sup>	0	ND	--
C-5030	0	ND	--
C-5030	0	ND	--
C-4638 <sup>2</sup>	0	ND	--
C-4638	0	ND	--
C-4638	0	ND	--
C-4570 <sup>3</sup>	0	ND	--
C-4570	0	ND	--
C-4570	0	ND	--
C-5030+S	0.0157	0.0075	48
C-5030+S	0.0157	0.0106	67
C-5030+S	0.0157	0.0119	75
C-4638+S	0.0157	0.0117	74
C-4638+S	0.0157	0.0106	67
C-4638+S	0.0157	0.0104	66
C-4570+S	0.0157	0.0111	71
C-4570+S	0.0157	0.0097	61
C-4570+S	0.0157	0.0105	66

Mean Recovery: 66  
Standard Deviation: 8.07

1. C-5030 is a control soybean sample grown at Athens, GA in 1982.
2. C-4638 is a control soybean sample grown at E. Lansing, MI in 1982.
3. C-4570 is a control soybean sample grown at Arlington, WI in 1982.

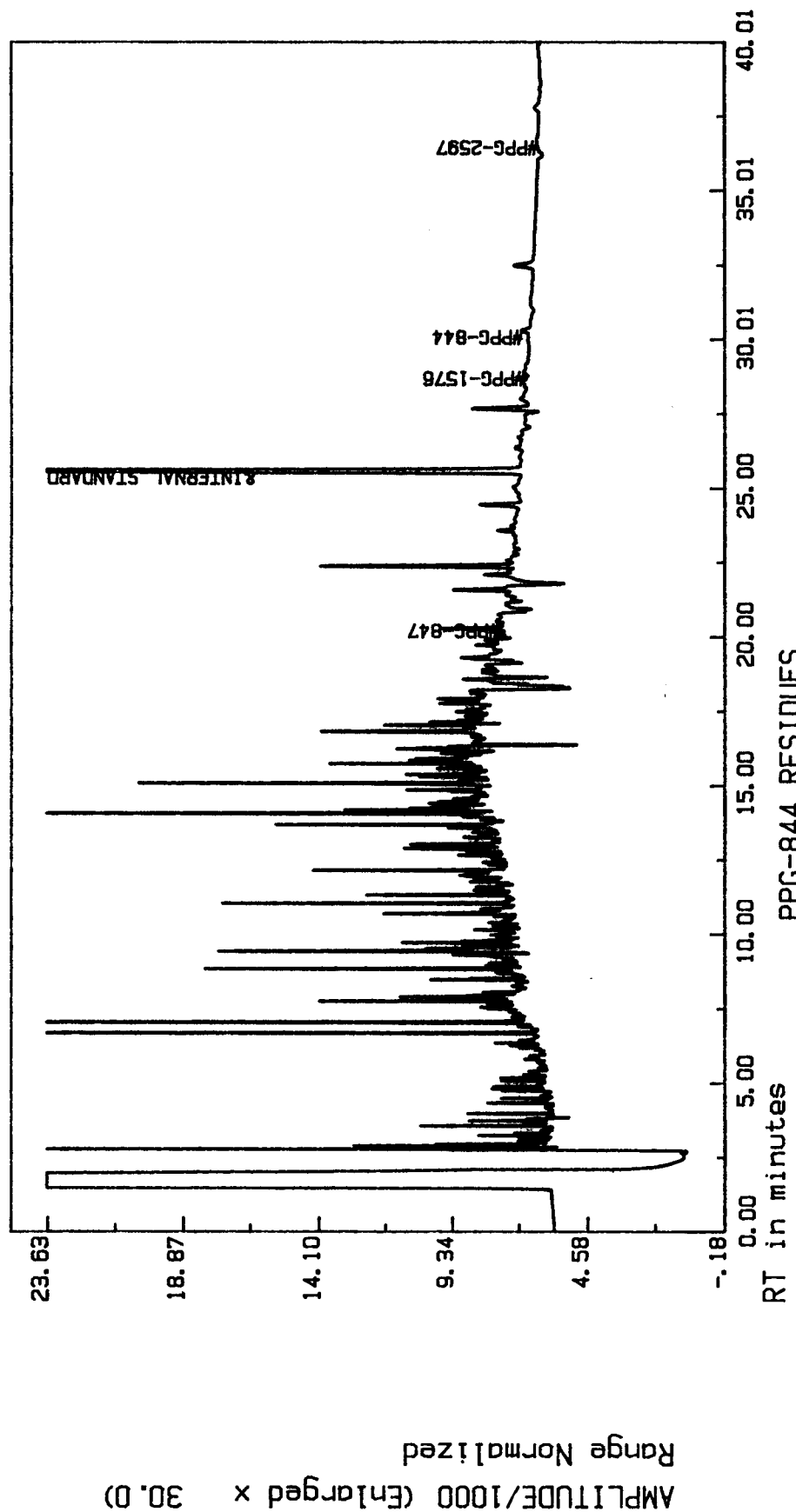
Analyzed 4/17/86, Notebook 6694, p. 229.

FIGURE 1. CALIBRATION STANDARD  
EACH METABOLITE ca. 1 pg/uL



PPG-844 RESIDUES  
SAMPLE: STD 35-3 INJECTED AT 17:21:24 ON FEB 26, 1987  
Meth: DB5 Raw: RQ4234 Proc: PQ4234

FIGURE 2. CONTROL SOYBEAN GRAIN  
ELANCO, LONDON, ONT. CANADA



SAMPLE: D 4910  
Meth: DB5

PPG-844 RESIDUES  
INJECTED AT 22:13:57 ON FEB 26, 1987  
Raw: RQ4238  
Proc: PQ4238



FIGURE 3. SPIKED SOYBEAN GRAIN  
EACH METABOLITE SPIKED AT ca. 10 ppb

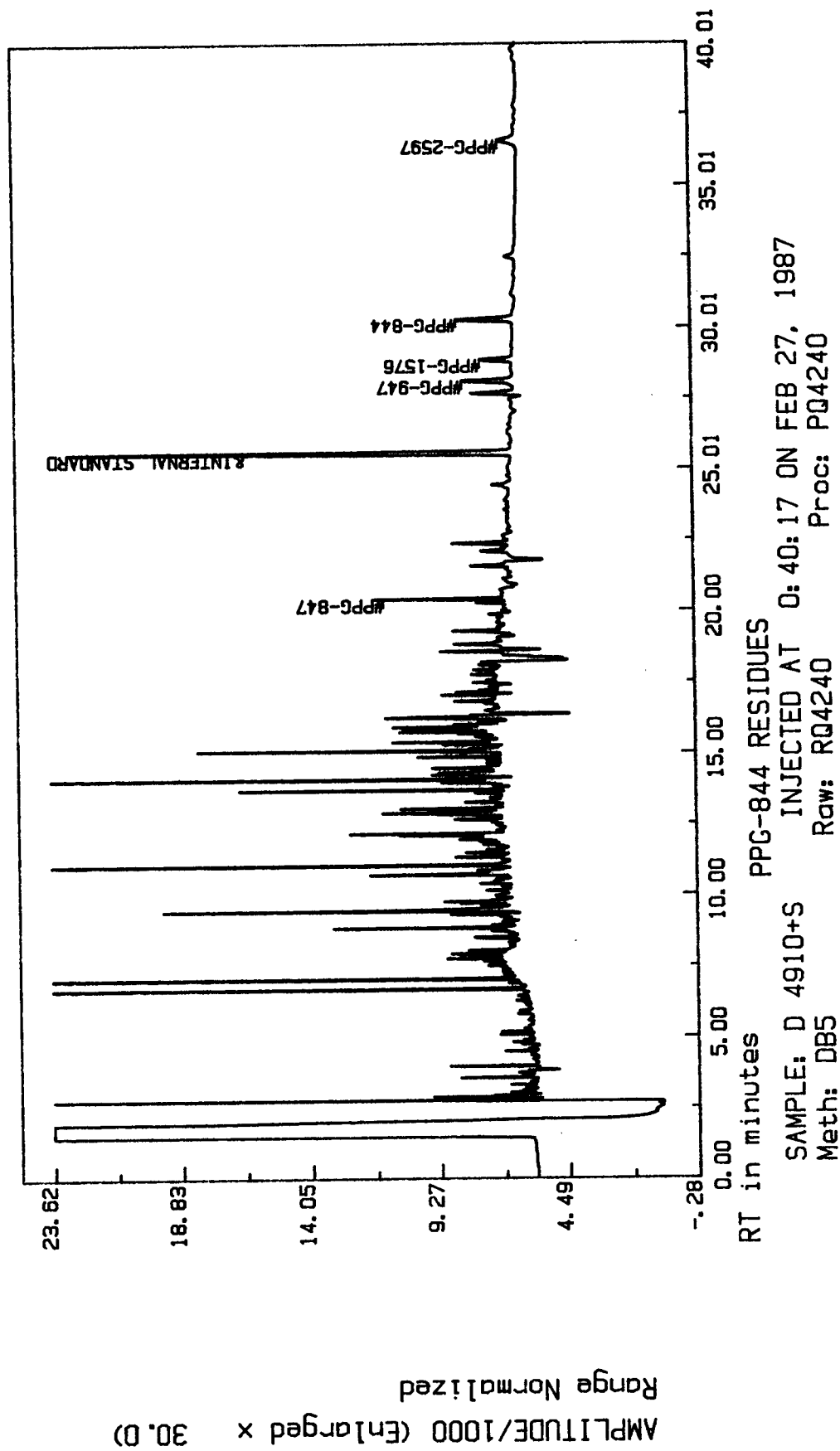
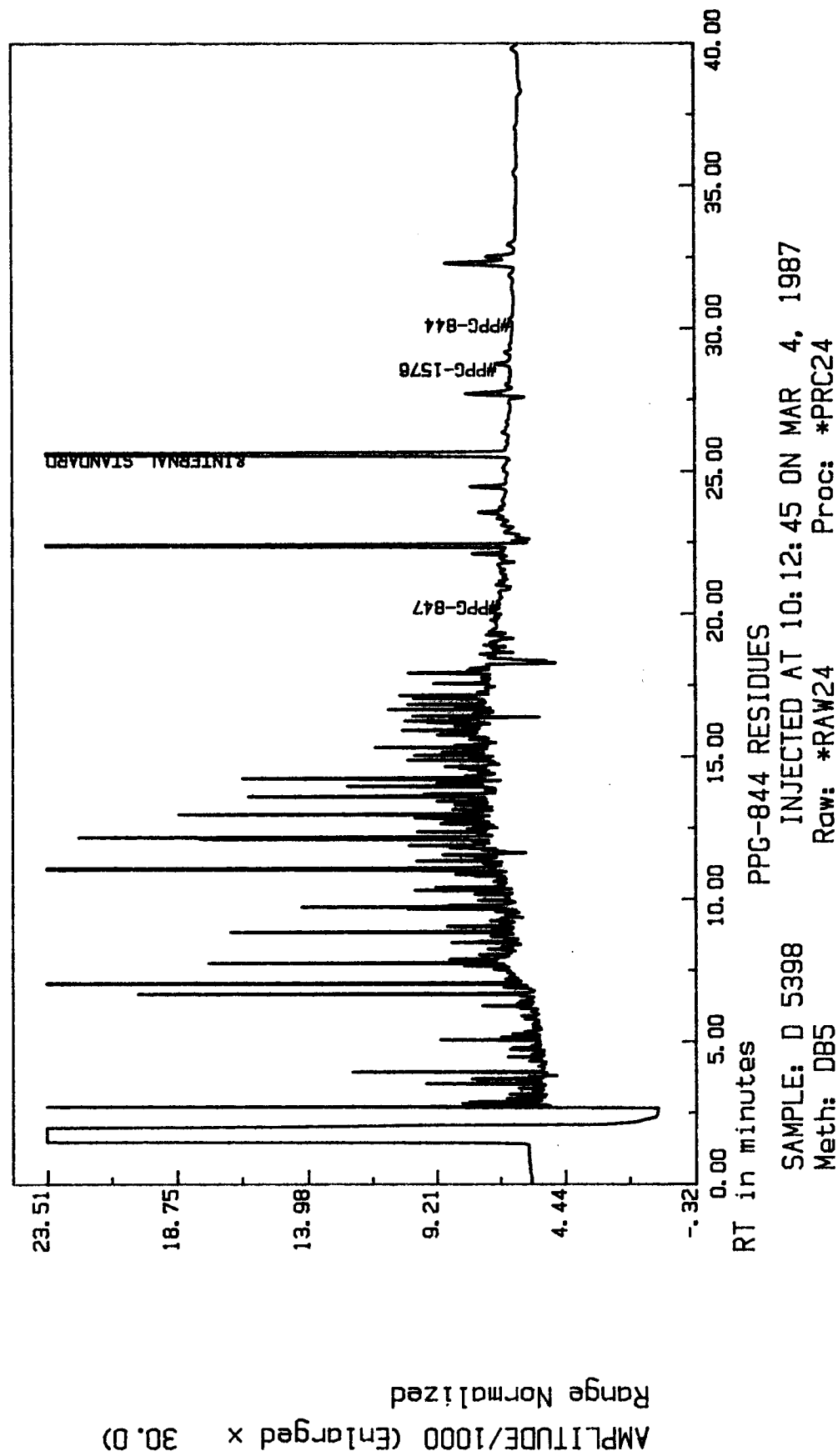


FIGURE 4. TREATED SOYBEAN GRAIN  
0.16#COBRA/A, BRAZIL



## APPENDIX I

\*\*\*MAR 4, 1987 10:08  
METHOD: DB5 ON CRN 13  
CHANNEL 24

CREATED: 10:11 ON MAY 16, 1983  
MODIFIED: 10:35 ON MAR 3, 1987

### 1. DATA INPUT

RUNTM #PKS  
40.00, -100

MU/MIN DELAY MIN-AR BUNCH  
.300, 18.00, 1000, AUTO

#### INTEGRATOR EVENTS

	TIME	EVENT
1	18.00,	R
2	28.00,	I
3	36.40,	I
4	/E	

#### CONTROL EVENTS

	TIME	EVENT	ECM	RLY
1	/E			

### 2. DATA ANALYSIS

PROC RPRT SUP-UNK  
ISTD, EX, NO

UNITS TITLE  
PPB , PPG-844 RESIDUES

REF-RTW %RTW RF-UNK ID-LVL DUT  
.050, .500, 0.0000E+ 0, 0, 0.00

CALIBRATION PEAKS CALIBRATED: 7:04 ON MAR 3, 1987

	TIME	AMOUNT	FACTOR	NAME
1	20.43,	1.5000E+ 1,	3.3877E+ 1,	#PPG-847
2	25.61,	1.0000E+ 0,	1.0000E+ 0,	&INTERNAL STANDARD
3	28.15,	1.5000E+ 1,	7.2532E+ 1,	#PPG-947
4	28.89,	1.5000E+ 1,	5.2592E+ 1,	#PPG-1576
5	30.33,	1.5000E+ 1,	4.2594E+ 1,	#PPG-844
6	36.62,	1.5000E+ 1,	6.7840E+ 1,	#PPG-2597
7	/E			

### 3. USER PROGRAMS

POST-ANAL DIALG-PRG PARAM-FILE  
/N

### 4. REPORTS

RDUC #RPTS  
1 L3, 1  
2 T5, 1  
3 /E